IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Bastian et al.	
U.S. Serial No.:	09/536,736	ART UNIT: 1636
Filed:	March 28, 2000	EXAMINER: G. Guidry, Ph.D.
Entitled:	Method for Isolating and Purifying Nucleic Acids on Surfaces	

Attorney Docket No.: QGN-009.1 US

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration of Uwe Oelmüller Pursuant to 37 CFR § 1.132

I, Uwe Oelmüller, hereby declare and state that:

1. I am the same Uwe Oelmüller named as co-inventor on this patent application. I currently hold the position of Director of Research & Development, Diagnostic Sample Preparation, at Qiagen GmbH (Hilden, Germany, assignee of the present application), where I have been employed since 1995. I have been involved in the field of isolation and purification of nucleic acids from biological sources for over 15 years. A copy of my curriculum vitae is attached at Tab A.

2. My research includes the development of new technologies that improve the isolation and purification of biomolecules, such as nucleic acids, including the development of a method for isolating and purifying nucleic acids on porous, non-siliceous membranes in a manner that involves binding nucleic acids to one side of a porous, non-siliceous membrane, followed by release and retrieval of the formerly bound nucleic acids from the same side of the membrane to which the nucleic acids were bound, such that the bound nucleic acids do not pass through to other side of the membrane.

3. On information and belief, I understand that claims of the present application to a process for isolating nucleic acids have been rejected as being obvious over primarily European Patent application No. 0 431 905 A1 (hereinafter "Ogawa") and further in view of Pfister et al., *J. Biol. Chem., 271(3)*: 1687-1694 (1996) (hereinafter "Pfister") and U.S. Patent No. 5,234,809 (hereinafter "the Boom patent"). I further understand that Pfister is used as an example of the RNeasy® kit and protocols for isolating nucleic acids (Qiagen GmbH, Hilden, Germany). I am familiar with and understand the primary reference Ogawa, Pfister, the RNeasy® kit and protocols, and the Boom patent.

4. I make this declaration to explain that the method for isolating and purifying nucleic acid from a sample described in the present application is distinctly different from the ultrafiltration process employed by Ogawa, the RNeasy® kit and protocols, and/or methods described in the Boom patent.

5. Ogawa describes the purification of phage DNA using ultrafiltration. Although other factors (e.g., molecular shape, charge) may play some role in the process, ultrafiltration separates molecules primarily on the basis of size (size discrimination, size exclusion), i.e., on the basis of whether or not a molecule is sufficiently small to pass through the pores of a particular ultrafiltration membrane (ultrafilter) or sufficiently large not to pass through the pores of the membrane. Thus, ultrafiltration can only purify nucleic acid molecules from other types of molecules, e.g., proteins, lipids, carbohydrates, in a complex mixture if the nucleic acid in the mixture is larger than the pores of a chosen ultrafiltration membrane and the other types of molecules in the mixture are sufficiently small to pass through the pores of the same ultrafiltration membrane.

6. In the case of Ogawa, phage DNA molecules released from phage particles are retained on an ultrafiltration membrane surface because the DNA molecules are too large to pass through the pores of the membrane, whereas smaller molecules, such as decomposed and denatured proteins, are sufficiently small to pass through the membrane pores. (See, Ogawa at column 4,

lines 23-34.) Clearly, if the size of the pores of the ultrafiltration membrane were sufficiently large, the phage DNA would also pass through the membrane with the other molecules, and no purification of the desired (target) DNA would be achieved. According to Ogawa, ultrafiltration membranes that are useful in the method of isolating phage DNA preferably have a fractionation molecular weight of 20,000 to 1,000,000 (daltons). (See, Ogawa at column 3, lines 31-34.) Ogawa provides an example of using such an ultrafiltration membrane to isolate M13 phage DNA. (See, Ogawa at column 4, lines 11-39.) In the example, cells of the bacterium *E. coli* were infected with M13 phage, and the cultures incubated for 4 hours at 37°C. The resulting culture medium containing phage particles and bacterial cells was then passed through a membrane having a pore size of 0.45 μm to obtain a cell-free sample (filtrate) of phage particles. The cell-free filtrate containing phage particles was then applied to an ultrafiltration membrane having a fractionation molecular weight of 300,000 (daltons), and the proteins of the phage capsids were denatured and decomposed with proteinase K. The denatured and decomposed proteins were washed through the ultrafiltration membrane along with other lower molecular weight molecules while the larger phage genomic DNA released from the phage particles was retained on the surface of the ultrafiltration membrane and subsequently retrieved in 200 microliters of TE buffer. Ogawa reports that this procedure recovered 2.5 μg of phage DNA from 2 milliliters of culture medium.

7. The selection of an appropriate ultrafiltration membrane for use in a particular separation or isolation procedure requires knowledge and consideration of the approximate size of the desired target molecule and that of the undesired molecules that may be present in a particular sample. In addition, manufacturers of commercially available ultrafiltration membranes have typically provided guidance in their catalogs to assist the practitioner in this field in the selection of an ultrafiltration membrane that is best suited for a particular procedure. For example, the website of the Pall Corporation (a major manufacturer and supplier of ultrafiltration membranes used in analytical and preparative protocols) provides a helpful section entitled "Ultrafiltration Fundamentals", which includes a background review of ultrafiltration, a description of various ultrafiltration devices, and guidelines for selecting an ultrafiltration membrane with the appropriate molecular weight cutoff (MWCO). (See, pages 1-6, "Ultrafiltration Fundamentals",

printed from the Pall Corporation website at http://www.pall.com/34696_35486.asp, attached at Tab B.) Tables 2 and 3 in Tab B can be used in selecting an ultrafiltration membrane for isolating DNA and separating out proteins. Table 2 indicates that an ultrafiltration membrane that has a MWCO of 300,000 or 1,000,000 would be useful for retaining various sizes of nucleic acid molecules, including nucleic acids in the size range of the single-stranded genomic DNA of M13 phage isolated in the example of Ogawa (see, above). Table 3 also indicates the nominal pore sizes of ultrafiltration membranes with various MWCO for proteins. According to Table 3, an ultrafiltration membrane with an MWCO of 1,000,000 has a nominal pore size of 100 nm (i.e., 0.100 μm), and an ultrafiltration membrane with an MWCO of 300K (300,000 daltons) has a nominal pore size of 35 nm (0.035 μm) and will effectively retain biomolecules that have a molecular weight in the range of 900,000 to 1,800,000 daltons. A consideration of these various guidelines indicates that the use of an ultrafiltration membrane with an MWCO of 300,000, as in Ogawa's example, would appear to be a reasonable choice for separating the single-stranded genomic DNA of an M13 phage from smaller denatured and decomposed phage proteins, which would wash through the pores of the ultrafiltration membrane.

8. A Pall Gelman Sciences catalog from 1998 also provides similar descriptions of ultrafiltration membranes that have been available for use in separating biomolecules. A table entitled "Relative Partikelgrößen" ("Relative Particle Size") from the 1998 catalog is provided at Tab C. The table shows the pore size in the column labeled "Å" (for angstroms) of recommended ultrafiltration membranes for separating biomolecules of the various sizes indicated in the column labeled "MG" (for molecular weight). The table indicates that fractionation of biomolecules having a molecular weight in the range of 300,000 daltons can be carried out using an ultrafiltration membrane with a pore size of about 350 angstroms, i.e., 0.035 μm, again in line with the ultrafiltration membrane employed in the example of Ogawa for separating M13 phage DNA from denatured and decomposed proteins. The table also indicates that use of ultrafiltration to separate out species in the size range of about 1,000,000 daltons from smaller molecules can be accomplished using an ultrafiltration membrane having a pore size of 1000 angstroms, i.e., 0.100 μm.

9. The table from the 1998 Pall Gelman Sciences catalog at Tab C also indicates that ultrafiltration membranes used in separating biomolecules have pore sizes in the range of about 0.001 μm (10 angstroms) to about 0.100 μm (1000 angstroms). For separating out larger species, other types of membrane filtration are employed. A Gram negative, rod-shaped bacterium, such as *E. coli*, is approximately 1.0 μm in diameter and 2.0 μm or more in length. Thus, membranes having pores sizes in the range of 0.22 μm to 0.45 μm are commonly employed to filter bacterial cells out of solutions or culture media as described in the example in Ogawa, in which a culture medium was passed through a membrane filter having a pore size of 0.45 μm in order to obtain a cell-free sample of phage particles (see, above).

10. The above comments and examples show that practitioners in the field of purification and isolation of biomolecules know that ultrafiltration membranes used to separate biomolecules have pores sizes of 0.100 μm or less, and that an ultrafiltration membrane that has a pore size of about 0.035 μm can be particularly useful for separating DNA molecules that are at least the size of M13 phage DNA from decomposed proteins and other smaller molecules. The selection of an appropriate ultrafiltration membrane for use in a particular protocol requires some knowledge of the approximate size of the desired target molecule and of the undesired molecules present in a sample. Membrane pore sizes greater than 0.100 μm are sufficiently large to permit most individual biomolecules, including DNA, RNA, and proteins, to pass through the membrane.

11. Being restricted to using ultrafiltration membranes, which have relatively small pore sizes (see, above), to isolate nucleic acids as described by Ogawa can be a disadvantage, especially when the goal is to purify nucleic acid from relatively complex biological samples, such as a crude cell lysate, because relatively small pores of an ultrafiltration membrane can easily become blocked or clogged with various impurities, such as cell fragments, organelles, and/or molecular aggregates that may be present in such samples. In addition, nucleic acids (DNA, RNA) can be trapped within impurities retained by ultrafiltration membranes. Thus, such complex samples typically require that one or more treatment steps be performed to remove such interfering impurities prior to ultrafiltration. In addition, many proteins are considerably larger than the 10,000 dalton MWCO of an ultrafiltration membrane having a pore size as small as 0.001 μm.

The yield and purity of DNA and/or RNA could therefore be higher if membranes having larger pore sizes could be used for isolating nucleic acids from such complex samples.

12. I and my co-inventors recognized that a process for isolating and purifying nucleic acids that permits use of porous, non-siliceous membranes that have pore sizes that are sufficiently large to allow cell fragments, proteins, and other undesired impurities to wash through while retaining the desired target DNA or RNA molecules on the same side of the membrane to which they were bound, would be a significant advantage over other processes, including previously known ultrafiltration methods, such as those employed in Ogawa. We invented the process described and claimed in the present application, in which DNA and/or RNA in a sample bind on one side of a porous, non-siliceous membrane. But in contrast to known ultrafiltration methods, such as employed in Ogawa, the process of our invention is not restricted to the relatively small (i.e., 0.100 μm or smaller) pore sizes of ultrafiltration membranes. In fact, our process typically provides relatively high yields of nucleic acids from cell lysates using membranes having much larger pore sizes, i.e., greater than 0.100 μm. By way of example, see, in the present application, Table 5 at page 25, where excellent yields of RNA were isolated from a HeLa cell lysate using membranes having pore sizes of 0.2 μm, 0.45 μm, 0.65 μm, 1.2 μm, 5 μm, 10 μm, and 20 μm.

13. From such data demonstrating that excellent yields of nucleic acids could be obtained even with non-siliceous membranes having pore sizes that well exceeded 0.100 μm, it was immediately evident to us, as it would be to those skilled in this field, that our process involved the preferential binding of nucleic acids to one side of such porous, non-siliceous membranes by a mechanism other than size discrimination as occurs in ultrafiltration, *for the reason that pore sizes above 0.100 μm are clearly larger than those employed in the capture of DNA or RNA molecules by simple ultrafiltration.* The larger pore sizes of the non-siliceous membranes that may be used in our invention would clearly not work in an ultrafiltration process as described by Ogawa as such pore sizes are also *significantly larger than the phage DNA separated by ultrafiltration in Ogawa.* Thus, it was surprising that our invention not only enabled us to isolate relatively large molecular weight DNA species (e.g., mammalian genomic DNA as in Examples

6 and 7 of the present application) but even much smaller RNA molecules from a variety of samples (see, e.g., Examples 1-5 of the present application).

14. Thus, according to our invention, both DNA and even smaller RNA molecules can be isolated using membranes that have such relatively large pore sizes. Although we do not intend to limit our invention by any particular theory of operation, we have speculated that in our method the nucleic acids are bound to the membrane material by direct interaction and are kept bound during washing steps. For eluting the nucleic acids, water or low salt buffers are used for releasing the nucleic acids from the membrane material. We have speculated that the surface tension of the water or the low salt buffer prevents the nucleic acid-containing eluate from dripping through the membranes, thus allowing retrieval of the nucleic acids from the same side of the membrane from which they were applied to the membrane.

15. In conclusion, our data presented in the application convincingly demonstrate that our method for isolating and purifying nucleic acids is not a form of ultrafiltration as employed by Ogawa. Persons in this field would have no confusion in distinguishing our process as demonstrated by our data and described in the present application from the well known process of ultrafiltration that involves size discrimination of biomolecules.

16. I am also familiar with Qiagen's RNeasy® products and protocols for isolating RNA, e.g., as employed in the Pfister reference. As explained in the RNeasy® Mini Handbook (1999), this product employs the *selective binding properties of a silica-gel-based membrane for RNA* with the convenience of microspin columns. The selectively bound RNA is ultimately eluted from and passed through the silica-gel-based membrane into the bottom of a microspin tube. The RNeasy® spin columns contain a silica membrane layer of about 1.5 millimeters (mm) thickness. The silica membrane in the RNeasy® spin columns is a fluffy, fibrous, fleece-like structured material. Liquid volumes used for the RNA elution get sucked into the membrane material when pipetted onto the membrane. This makes it impossible to harvest the eluate from the membrane surface by simple means such as pipetting. Consequently, the RNeasy® Mini Handbook describes how to elute the RNA by passing the RNA-containing eluate through the

silica membrane by centrifugation. This is clearly different from our invention where the nucleic acid-containing eluate does not get sucked into the membrane but is kept on the membrane surface. In addition, Example 1 of the present application shows that a fleece-like silica membrane (which, in fact, was the silica gel-based membrane material used in RNeasy® spin columns) did not permit retrieval of high levels of RNA from the same side of the material to which the RNA was applied. The specification further states that such ineffective performance was presumably due to the fleece-like structure and the ensuing adsorption of a large portion of the eluate buffer. (See, in the specification, page 17, lines 16-17 and Table 1.) Persons skilled in this field would clearly distinguish the specific and selective features of the silica-gel-based RNeasy® products and protocols from the methods of our invention for isolating nucleic acids (DNA and/or RNA), which employs highly porous, non-siliceous membranes as described in the present application. Moreover, persons skilled in this field would not consider the RNeasy® products and protocols as providing a basis for modifying the ultrafiltration method of Ogawa to form our method for isolating and purifying nucleic acids according to the present application.

17. I am also familiar with the methods in the Boom patent in which cells are lysed using a chaotropic agent, such as guanidinium thiocyanate, and then bound to silica coarse particles (silicon dioxide particles) or non-siliceous materials. Note that according to the methods of the Boom patent, nucleic acid can be bound and eluted from the silica particles, but it was thought that bound nucleic acids could not be eluted from any non-siliceous membrane. (Note -- "[N]ormally no release of DNA takes place in the low salt buffer", column 22, lines 8-9, of the Boom patent.) The Boom patent therefore teaches inserting a filter with DNA bound to it directly into the PCR assay mixture. (See, column 22, lines 8-10, of the Boom patent.) This feature alone demonstrates a clear difference between the methods of the Boom patent and that of our invention in which purified nucleic acids are retrieved in high yields from the same side of a highly porous, non-siliceous membrane to which they are introduced in a sample.

18. The comments and information presented herein illustrate the clear differences in the method for isolating and purifying nucleic acids using porous, non-siliceous membranes as described in the present application and the ultrafiltration process employed by Ogawa, even

considering also the selective RNA binding property of a silica-gel employed in RNeasy® products and protocols, and the guanidinium-dependent, irretrievable binding of nucleic acids to non-siliceous membranes described in the Boom patent. Accordingly, persons skilled in this field would have no confusion in distinguishing the process of the present application from these other methods.

19. I further declare that all statements made herein of my own knowledge are true and that statements made upon information and belief are believed to be true and further that false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

05/15/07
date

Uwe Oelmüller, Ph.D.

CV Dr Uwe Oelmüller

1986 **Diploma Degree in Biology**

1991 **Doctor Degree in Natural Science at the Institute for Microbiology at the University of Goettingen, Germany**

- Development of methods for the isolation of bacterial RNA
- Analysis of the stability of mRNAs in bacteria
- Processing of mRNAs in bacteria

1991 – 1995 **Post Doc at the University Hospital Göttingen, Germany**

- Transcript profile of HIV-1 in infected patients
- Analysis of proviral HIV-1 genomes

1995 – 1997 **QIAGEN GmbH: laboratory manager**

- Heading a team of 2 – 4 technicians
- Development of membrane based RNA and genomic DNA isolation technologies
- Development of ion change chromatography based RNA isolation methods
- Launch of five products for the isolation of total RNA and mRNA from biological and clinical samples

1997 – 2000 **QIAGEN GmbH: project manager**

- Heading a team of 10 employees (scientists, technicians)
- Development of membrane and cationic detergent based technologies for the isolation of cellular RNA, genomic DNA and viral nucleic acids
- Development of technologies for the stabilization of RNA and DNA profiles in biological samples
- Launch of various products for the isolation of RNA, DNA and viral nucleic acids from biological and clinical samples

2000 – 2002 **QIAGEN GmbH: Associate Director R&D**

- Heading the technology center for RNA isolation and nucleic acid stabilization
- Heading a team of 17 employees (scientists, technicians)
- Development of products for the collection, stabilization of clinical samples and the isolation of RNA and DNA from these samples
- Development of automated solutions for the isolation of cellular RNA from various samples materials in high throughput formats
- Developments based on various technologies for the stabilization/isolation of nucleic acids: silica and other membrane

technologies, detergent based technologies, salting out, ion exchange, hybrid capture etc.

2002 – now **QIAGEN GmbH: Director R&D Diagnostic Sample Preparation**

- Worldwide responsibility for QIAGEN's diagnostic sample preparation product developments
- Heading a team of currently 46 employees
- Development of products for the collection, stabilization of clinical samples and isolation of all kind of nucleic acids from these samples
- Development of in vitro diagnostic products for the collection, stabilization of clinical samples and isolation of RNA, DNA and pathogen nucleic acids from various samples types
- Standardization of complete workflows from clinical sample collection to nucleic acid isolation and to the final assay detection

1999 – 2006 **Scientific Director of PreAnalytiX, a joint venture between QIAGEN and Becton Dickinson**

- Responsibility for QIAGEN's R&D within the PreAnalytiX joint venture
- Launch of several research and medical products for the collection and stabilization of the cellular RNA profile in human blood and isolation of cellular RNA and genomic DNA
- Various international co-operations in the field of nucleic acid stabilization and isolation

2006 – now **Management Committee Member of PreAnalytiX, representing the company's R&D**

- Responsibility for the company's R&D program
  - Technology developments for the stabilization of clinical samples and isolation of nucleic acids from clinical samples
  - Medical and research product developments
- Various international co-operations

- Responsibility for the launch of more than 50 QIAGEN products for the stabilization of clinical samples and the isolation for nucleic acids within QIAGEN's worldwide well known product lines of RNeasy, QIAamp, QIAzol, QIAGEN RNA/DNA, Oligotex, BioRobots MDx, M48 and EZ1 and for PreAnalytiX's product line PAXgene.

- More than 10 patent applications in the field of sample collection, nucleic acid stabilization, and nucleic acid isolation

- 6 international publications in the field of nucleic acid isolation and nucleic acid analysis

- Various presentations at international meetings and congresses in the field of nucleic acid analysis

Ultrafiltration Fundamentals

Background

Ultrafiltration (UF) is a membrane separation technique used to separate extremely small particles and dissolved molecules in fluids. The primary basis for separation is molecular size, although other factors such as molecule shape and charge can also play a role. Molecules larger than the membrane pores will be retained at the surface of the membrane (not in the polymer matrix as they are retained in microporous membranes) and concentrated during the ultrafiltration process.

Compared to non-membrane processes (chromatography, dialysis, solvent extraction, or centrifugation), ultrafiltration:

- is far gentler to the molecules being processed
- does not require an organic extraction which may denature labile proteins
- maintains the ionic and pH milieu
- is fast and relatively inexpensive
- can be performed at low temperatures (for example, in the cold room)
- is very efficient and can simultaneously concentrate and purify molecules.

The retention properties of ultrafiltration membranes are expressed as Molecular Weight Cutoff (MWCO). This value refers to the approximate molecular weight (MW) of a dilute globular solute (i.e., a typical protein) which is 90% retained by the membrane. However, a molecule's shape can have a direct effect on its retention by a membrane. For example, linear molecules like DNA may find their way through pores that will retain a globular species of the same molecular weight.

There are three generic applications for ultrafiltration:

1. **Concentration**. Ultrafiltration is a very convenient method for the concentration of dilute protein or DNA/RNA samples. It is gentle (does not shear DNA as large as 100 Kb or cause loss of enzymatic activity in proteins) and is very efficient (usually over 90% recovery).

2. **Desalting and Buffer Exchange (Diafiltration).** Ultrafiltration provides a very convenient and efficient way to remove or exchange salts, remove detergents, separate free from bound molecules, remove low molecular weight materials, or rapidly change the ionic or pH environment.
3. **Fractionation.** Ultrafiltration will not accomplish a sharp separation of two molecules with similar molecular weights. The molecules to be separated should differ by at least one order of magnitude (10X) in size for effective separation. Fractionation using ultrafiltration is effective in applications such as the preparation of protein-free filtrates, separation of unbound or unincorporated label from DNA and protein samples, and the purification of PCR products from synthesis reactions.

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Choosing the Correct Device

Devices are available in a range of sizes that accommodate the following sample volumes (Table 1):

Table 1
Device Selection Based on Volume Filtered

Sample Volume	Use
50 µL to 500 µL	Nanosep® Device
500 µL to 3.5 mL	Microsep™ Device
3 mL to 15 mL	Macrosep® Device
15 mL to 60 mL	Jumbosep™ Device

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Choosing the Correct MWCO

Once sample volume is determined, the next step is to select the appropriate MWCO (for ultrafiltration) or pore size (for microfiltration). MWCOs are nominal ratings based on the ability to retain > 90% of a solute of a known molecular weight (in Kilodaltons). Table 2 and 3 provide retention characteristics of different MWCO membranes for some solutes. For proteins, it is recommended that a MWCO be selected that is 3 to 6 times smaller than the molecular weight of the solute being retained. If flow rate is a consideration, choose a membrane with a MWCO at the lower end of this range (3X); if the main concern is retention, choose a tighter membrane (6X).

It is important to recognize that retention of a molecule by a UF membrane is determined by a variety of factors,

among which its molecular weight serves only as a general indicator. Therefore, choosing the appropriate MWCO for a specific application requires the consideration of a number of factors including molecular shape, electrical charge, sample concentration, sample composition, and operating conditions. Because different manufacturers use different molecules to define the MWCO of their membranes, it is important to perform pilot experiments to verify membrane performance in a particular application.

Common variables that increase molecule passage:

- Sample concentration less than 1 mg/mL (Figure 1).
- Linear versus globular molecules.
- High transmembrane pressure created by g-force in centrifugal concentrators. (This is especially important in the case of linear molecules, for example DNA fragments. Decreasing the g-force can increase retention of molecules by a membrane.)
- Buffer composition that favors breakup of molecules.
- pH and ionic conditions that change the molecule (for example, cause conformational changes or aggregation).

Common variables that decrease molecule passage:

- Sample concentration higher than 1 mg/mL.
- Buffer conditions that permit molecules to aggregate.
- Presence of other molecules that increase sample concentration.
- Lower transmembrane pressure. (In the case of centrifugal concentrators, lower g-force.)
- Adsorption to the membrane or device.
- Low temperature (4 °C versus 24 °C).

Table 2
MWCO Selection for Nucleic Acid Applications

MWCO	Base Pairs (DS)	Bases (SS)
1K	5 - 16 Bp	9 - 32 Bs
3K	16 - 32 Bp	32 - 65 Bs
5K	25 - 50 Bp	50 - 95 Bs
10K	50 - 145 Bp	95 - 285 Bs
30K	145 - 285 Bp	285 - 570 Bs
50K	240 - 475 Bp	475 - 950 Bs

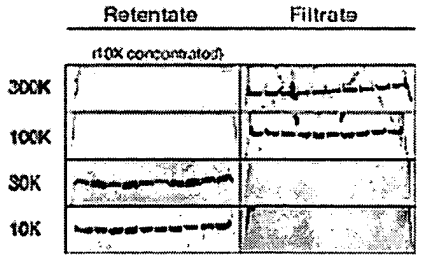
100K	475 - 1,450 Bp	950 - 2,900 Bs
300K	1,450 - 2,900 Bp	2,900 - 5,700 Bs
1,000K	4,800 - 9,500 Bp	> 9,500 Bs

Table 3
MWCO Selection for Protein Applications

MWCO	Membrane Nominal Pore Size	Biomolecule Size	Biomolecule Molecular Weight
1K			3K - 10K
3K			10K - 20K
5K			15K - 30K
10K			30K - 90K
30K			90K - 180K
50K	5 nm	15 - 30 nm	150K - 300K
100K	10 nm	30 - 90 nm	300K - 900K
300K	35 nm	90 - 200 nm	900K - 1,800K
1,000K	100 nm	300 - 600 nm	> 3,000K

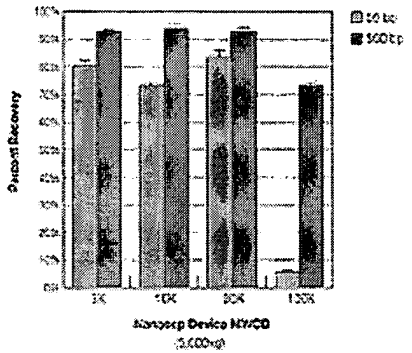
Figure 1
Retention and Passage of Dilute Protein Samples

Ten 400 µL samples of a dilute solution of BSA (100 ng/mL) were centrifuged in the indicated MWCO Nanosep devices. Retentate was recovered in 40 µL of phosphate buffered saline (10X concentrated relative to the original sample). 20 µL



aliquots of the retenate and filtrate from each device were denatured and electrophoresed on a 4 to 20% polyacrylamide gel and stained with coomassie blue. At higher initial concentrations, some BSA would normally be retained by the 100K device (data not shown) while at an initial concentration of 100 ng/mL, no BSA was detected in the retentate.

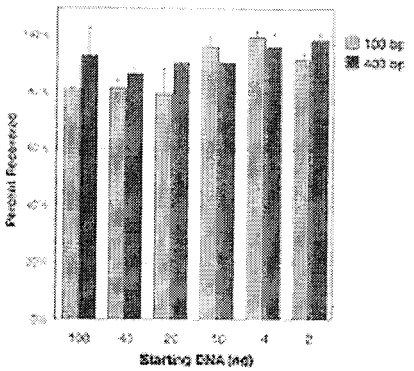
Figure 2a
Retention and Passage of Dilute Protein Samples



Click graph to enlarge

A 500 µL sample of a 100 µg/mL DNA solution containing 50 bp and 500 bp double-stranded DNA fragments was centrifuged at 5,000 x g in Nanosep devices to a final volume of 50 µL. Recovered samples were quantitated using absorbance at 260 nm. We found that the 100K device is able to separate these fragments; the 50 bp fragment was recovered in the filtrate, and the 500 bp fragment was recovered in the retentate.

Figure 2b
Recovery of Dilute DNA Samples



Click graph to enlarge

Nanosep 30K devices were used to filter dilute radioactive DNA fragments. In order to accurately quantitate DNA recovery from dilute samples, PCR products (100 and 400 bp) were dual labeled to low-specific activity with ^{32}P-dCTP and ^{32}P-dATP and prepared for filtration. After synthesis, unincorporated nucleotides as well as termination products were removed by ultrafiltration using a 30K Nanosep device. The resulting retentate was checked for size and quantitated using gel electrophoresis. Labeled DNA in quantities ranging from 100 ng all the way down to 2 ng per device was diluted to 500 µL using TE. The samples (in triplicate) were centrifuged at 5,000 x g for 10 minutes (spun to dryness) and recovered in two washes of 20 µL water. The resulting retentate was added to a counting vial containing scintillation solution and counted. Nanosep devices reliably recover small, dilute DNA samples.

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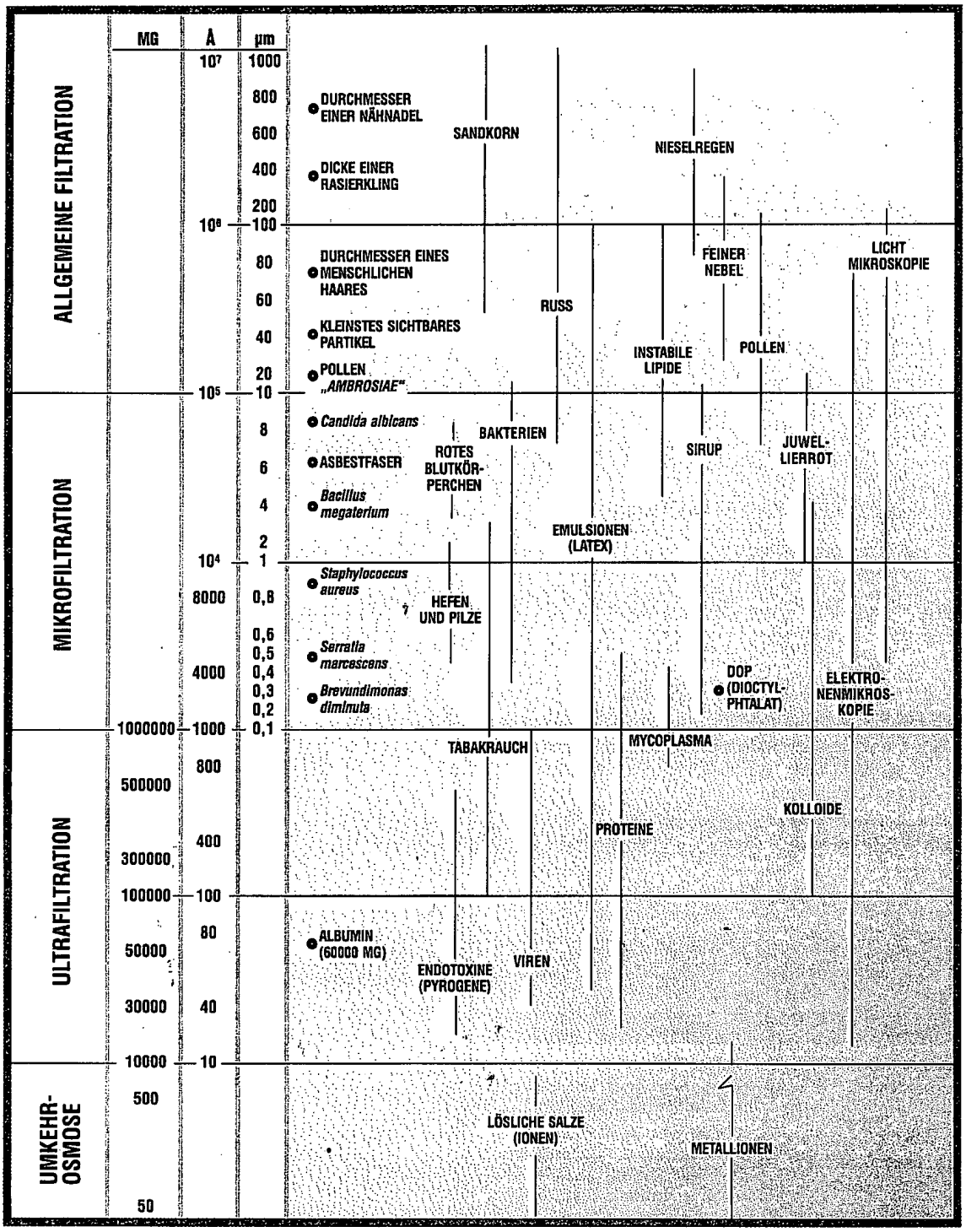
Relative Partikelgrößen



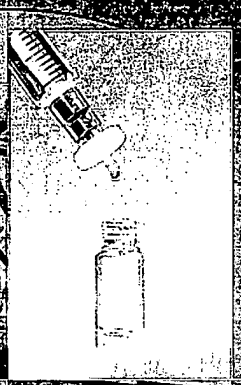
Å, ANGSTRØM = 10^{-8} cm µm, MIKROMETER (MIKRON) = 10^{4} Å 1 mil = 0,001 Zoll = 25,4 µm

Der Differenzdruck steigt mit abnehmender Porengröße. Die Schmutzhaltekapazität und die relative Fließrate sinken mit abnehmender Porengröße.

Für Bestellungen oder technische Informationen wenden Sie sich bitte an die Pall Gelman Sciences Niederlassung in Ihrer Nähe (siehe Seite 192).

www.gelman.com

PALL GelmanSciences



Das FilterBuch

Das umfassende Handbuch für Membranfiltration und Separation in Ihrem Labor

1998

PALL GelmanSciences

Das FilterBuch

Das umfassende Handbuch für Membranfiltration und -separation in Ihrem Labor

Willkommen zu unseren neuesten Filterhandbuch, einem einzigartigen Nachschlagewerk mit allen Produkten und Informationen, die Sie für Filtrations-, Trennungs- und Reinigungs aufgaben im Labor benötigen.

1997 ist ein aufregendes Jahr für Gelman Sciences, da unser Zusammenschluß mit der Pall Corporation Ihnen eine noch größere Auswahl modernster Technologien bietet, u. a. Produkte für die Ultrafiltration und Zentrifugenkonzentratoren von Filtron sowie Membranen von Pall.

Im Labor ist das Membranmaterial entscheidend für die Effektivität einer Filtration. Pall, Pall Gelman Sciences und Filtron sind anerkannte Marktführer in der Entwicklung neuer Membrantechnologien. Unser Angebot umfaßt 20 verschiedene Materialien in vielen hundert Filterkonfigurationen.

Für Wissenschaftler, die in der Entwicklung oder Qualitätssicherung für Pharmazie, Chemie oder andere Industrien arbeiten, ist die Filtration ein wichtiger Faktor. Die gleichen Filter, die im Entwicklungs- oder Analysestadium benutzt werden, sind jetzt auch für das „Scale Up" bis zum Produktionsmaßstab verfügbar. Dies garantiert der weltweit führende Hersteller von Produkten für Filtration und Membrantrenntechnik, die Pall Corporation.

Nicht geändert hat sich unsere Auffassung, daß Zuverlässigkeit, Beständigkeit und Leistung unserer Produkte sowie die Qualität der technischen Unterstützung und des Kundendienstes für uns eine Verpflichtung sind. Unser Beitritt zur Pall-„Familie" bietet Ihnen einen noch besseren Zugang zu den qualifizierten und erfahrenen technischen Spezialisten.

Kontinuierliche Innovationen und Verbesserungen der Produkte – das ist unser Ziel. Bei der Realisierung von Produkten und Dienstleistungen orientieren wir uns ständig an Ihren sich wandelnden Anforderungen, um Ihre Arbeit leichter und produktiver zu gestalten. Sollten Sie Anmerkungen, Fragen oder Empfehlungen für mögliche Verbesserungen unserer Produkte und Leistungen haben, so wenden Sie sich bitte an Ihre nächstgelegene Pall Gelman Sciences Niederlassung oder rufen Sie uns direkt in den USA an: (001) (734) 913-6197.

Garantie für Qualität und Leistung

Für jedes Produkt in diesem Katalog geben wir Ihnen die Garantie, daß es mit den besten Materialien, nach den höchsten Produktionsstandards und unter Anwendung der strengsten Qualitätssicherungsmaßnahmen hergestellt wurde. Die Qualitätssicherungssysteme der Fertigungsstätten von Pall Gelman Sciences sind nach ISO 9001 zertifiziert.

Bitte wenden Sie sich mit allen Fragen zu den von Ihnen verwendeten Produkten von Pall Gelman Sciences an die Vertriebsniederlassung in Ihrer Nähe. Wir wollen, daß Sie mit uns absolut zufrieden sind.

Membranen

PALL GelmanSciences

Die Herstellung von mikro- und ultraporösen Membranfiltern stehen im Mittelpunkt von Pall Gelman Sciences. Unsere Membranen werden mit modernsten Fertigungsanlagen produziert. Vom verwendeten Polymer bis zur exakten Porengröße – immer ist es die Membran, die den Wirkungsgrad eines Filters bestimmt.

Was ist eine Membran?

Eine Filtermembran ist ein dünner Polymerfilm mit vielen tausend mikroskopisch kleinen Poren. Die Größe dieser Poren bestimmt die Abscheiderate der Membran. Membranen können zur quantitativen Trennung oder Filtration schwebender Stoffe aus Flüssigkeiten und Gasen verwendet werden.

Membranen haben viele verschiedene Eigenschaften, die das Verhalten des Filters in den Anwendungen beeinflussen. Diese Eigenschaften werden im Abschnitt „Grundlagen der Filtration" ab Seite 10 ausführlich erläutert.

Pall Gelman Sciences bietet eine äußerst umfangreiche Produktpalette von Membranen für wissenschaftliche Untersuchungen und Tests auf den Gebieten Biotechnologie, Analytische Chemie, mikrobiologische Analysen und Probennahme im Umweltschutz an. Der vorliegende Abschnitt des Kataloges nennt die Spezifikationen aller von Pall Gelman Sciences angebotenen Membranfilter.

Weitere anwendungsbezogene Hinweise finden Sie im Abschnitt „Produktübersicht" auf den Seiten 14-15.